Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Fumigant Activity of Eight Plant Essential Oils Against Workers of Red Imported Fire Ant, *Solenopsis invicta*

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Article History
Edited by:
Gilberto M. M. Santos, UEFS, Brazil
Received 06 December 2012
Initial acceptance 16 February 2013
Final acceptance 05 March 2013

Keywords
Invasive ant, *Solenopsis invicta*, Control, Plant essential oil, Fumigation toxicity

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Abstract

Plant essential oils from eight plant species were tested for their insecticidal activities against the red imported fire ant, *Solenopsis invcita*, by using a fumigation bioassay. This study reveals that the mortalities after treatment of the workers of red imported fire ants varied according to the classification of workers, oil type, dosage, and exposure time. Among the essential oils tested, strong insecticidal activity was observed with the essential oils of camphor (*Cinnamomum camphora* (L.) J. Presl), artemisia annua (*Artemisia annua* L.), eucalyptus (*Eucalyptus globulus* Labill.), mugwort (*Artemisia argyi* H. Lév. & Vaniot), and wintergreen (*Ilex chinensis* Sims). Ant mortalities from chrysanthemum oil (*Dendranthema indicum* (L.) Des Moul.), turpentine oil (*Pinus massoniana* Lamb.), and forsythia oil (*Forsythia suspense* (Thunb.) Vahl) treatments were significantly lower than those from the previously mentioned five essential oil treatments. This study showed that camphor, artemisia annua, eucalyptus, mugwort, and wintergreen oils may have potential to be used as substitutes for chemical insecticides.

Introduction

The red imported fire ant, *Solenopsis invcita* Buren, which causes severe damage to humans, animals, and the environment, is an important medical and agricultural pest in southern United States, as well as Australia, the Philippines, Taiwan, and Mainland China (Zhang et al., 2007). The ant stings humans, pets, farm animals, and wildlife, as well as damaging farm, electrical equipments and irrigation systems. Moreover, besides destroying crops and fruits directly or indirectly, they negatively affect the local biodiversity and cause nearly $5 billion losses in urban and agricultural areas yearly in the USA (Cheng et al., 2008).

Common methods for managing the red imported fire ant are through insecticides or baits that are also a threat to human health and the environment because of their high toxicity of chemicals (Vogt et al., 2002). Reducing the use of synthetic contact insecticides has gained increasing interest in the research and development of alternative control tactics, such as fire ant fumigants (Zhou et al., 2012).

Fumigation has a major function in insect pest elimination in stored products and in quarantine containers. Studies on the toxicity of botanical essential oils on red imported fire ant have shown that various essential oils are repellent and/or toxic to the ant. Citrus peels showed contact toxicity against red imported fire ants (Sheppard, 1984). Aromatic cedar mulch also showed repellent activity against red imported fire ants (Thorvilson & Rudd, 2001). Vogt et al. (2002) tested mound drench formulations containing raw citrus peel extract (orange oil) on fire ants and found that cold-press citrus peel extract ('orange oil') can be effectively used as an organic alternative to conventional insecticides for drenching colonies.

Mint oil granules were proven to be toxic and repel-

lent to red imported fire ants, and all red imported fire ant mounds that were treated with mint oil granules were abandoned (Appel et al., 2004). Citronella oil was repellent and toxic to both Argentine and red imported fire ants (Wiltz et al., 2007). The essential oil from *Cinnamomum osmophloeum* Kaneh. leaves was reported to be toxic to red imported fire ants in open and closed exposure trials (Cheng et al., 2008). Callicarpenal and intermedeol were isolated from the leaves of American beautyberry (*Callicarpa americana* L.) and Japanese beautyberry (*Callicarpa japonica* Thunb.), and had repellent effects on red imported fire ants (Chen et al., 2008).

The over-the-counter essential oil products from China FYJ were investigated for repellent effects against workers of red imported fire ants, and each of its six major components also showed repellent effects at various concentrations (Chen, 2009). Without prior exposure to air, vetiver oil showed a significant repellent effect to workers. However, the repellent effect of vetiver oil to previously exposed workers reduced at low concentrations (Li et al., 2009).

The repellent effects of five botanical essential oils, namely, *Cymbopogon nardus* (L.) Rendle, *Cinnamomum cassia* (L.) C. Presl, *Ilex pedunculosa* Miq., *Salvia sclarea* L., and *Capsicum annuum* L. on fire ant workers were evaluated using a Y-tube olfactometer bioassay (Wang et al., 2012). However, few researches existed on fumigant investigations related to plant essential oils against the red imported fire ant.

Zhou et al. (2012) tested the fumigant activity of methyl bromide and found that all red imported fire ants treated with methyl bromide above 22.52 g/m^2 for 8 h died. Plant essential oils of garlic (*Allium sativum* L.), glove bud (*Eugenia caryophyllata* Thunb.), ajowan (*Trachyspermum ammi* (L.) Sprague), allspice (*Pimenta dioica* (L.) Merrill), caraway (*Carum carvi* L.), dill (*Anethum graveolen*s L.), geranium (*Pelargonium graveolens* L' Hér.), litsea (*Litsea cubeba* (Lour.) Pers.) showed strong insecticidal activity after testing 59 oils on the Japanese termite, *Reticulitermes speratus* Kolbe, by using a fumigation bioassay (Park & Shin, 2005; Seo et al., 2009). Formic acid has been tested in the laboratory for contact and fumigation toxicity against workers, alates, and queens of red imported fire ants (Chen, 2012).

Because of being highly volatile, constituting a rich source of bioactive chemicals (Chang et al., 2001), being safe as they are commonly used as fragrances and flavoring agents for foods and beverages (Isman, 2000), plant essential oils were used as potential alternatives for chemical insecticides.

We evaluated the fumigant activity of eight plant essential oils on red imported fire ant workers with a fumigation bioassay to determine the essential oils with efficient fumigant activity to red fire ant.

Materials and Methods

Plant essential oils

Plant essential oils of camphor (*Cinnamomum camphora*), wintergreen (*Ilex chinensis*), eucalyptus (*Eucalyptus globulus*), artemisia annua (*Artemisia annua*), mugwort (Artemisia argyi), chrysanthemum (*Dendranthema indicum*), forsythia (*Forsythia suspense*), and turpentine (*Pinus massoniana*) were purchased from Kangshen Natural Medicinal Oil Refinery (Jiangxi, China), on January 12, 2012.

Insects

S. invicta colonies were collected from the suburb of Guangzhou and maintained in the laboratory for bioassays. The collected ants were fed with a mixture of 10% honey and live insects (*Tenebrio molitor* L.). A test tube (25 mm×200 mm), which was partially filled with water and plugged with cotton, was used as a water source. The ants were maintained in the laboratory at 25±2 C.

Fumigation toxicity bioassay

The method of Seo et al. (2009) was used with slight modifications to determine the effectiveness of controlling the red imported fire ant. A 1.5 mL centrifuge tube treated with the essential oil being tested was placed at the bottom lid of a glass cylinder (5 cm diameter×10 cm) with eight pin holes on the tube to allow the oil to vaporize into the bottle. The lid was then sealed. The vertical wall inside each glass cylinder was coated with a Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. We classified the fire ant workers as major workers (4.3 mm to 4.5 mm body-length, 1.0 mm to 1.1 mm head-width) and minor workers (2.8 mm to 3.0 mm body-length, 0.6 mm to 0.7 mm head-width). Fifteen fire ant workers were placed at the bottom lid of the glass cylinder. The insects were maintained at 25±1°C and at a relative humidity of 80%. Experiment 1 (Cumulative mortalities were determined 12 and 24 h after testing at the concentration of 0.5, 1, 2, 3, and 5 mg/centrifuge tube). Experiment 2 (The cumulative mortalities were then determined 5, 8, 11, 13, and 24 h after testing with camphor, wintergreen, eucalyptus, artemisia annua, and mugwort oils at a concentration of 2 mg/centrifuge tube). All treatments were replicated three times. We used the following equations:

$$\text{Cumulative mortality}(\%) = \frac{\text{Number of dead ants}}{\text{Total number of test ants}} \times 100$$

Statistical analysis

For effect of oil type, dosage and exposure time on mortalities of the minor and major workers, data were trans-

formed to arcsine square root values for three-way analysis of variance (ANOVA) at P = 0.05 for the significance of main effects and various interactions. Means were compared and separated by using the Duncan-test.

Results

When eight plant essential oils were bioassayed, the mortalities of minor and major workers varied significantly according to oil type, dosage, and exposure time (ANOVA, P < 0.05). In addition, all of the interactions among the three factors were found to be significant (Tables 1, 3). The results on mortality were shown in Table 2 and Table 4. Significant differences were found among workers: developing from different oil types, exposed to different time and different dosage treatments (df = 28, F =7.623, F = 9.074, P = 0.0001).

Table 1. Analysis of variance (three-way ANOVA) for main effects (oil type, dosage and exposure time) on mortality of minor workers, and interactions.

	Main effects			Interactions			
	O	D	T	O×D	O×T	D×T	O×D×T
df	7	4	1	28	7	4	28
F	224.339	734.465	628.156	14.829	42.245	13.402	7.623
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

0: oil type, D: dosage, T: exposure time (P=0.05; df=239).

A 100% mortality was achieved in five out of eight essential oils, namely, camphor, artemisia annua, eucalyptus, mugwort, and turpentine oils, 12 h after treatment at 5 mg/centrifuge tube to minor workers. Wintergreen, chrysanthemum, and forsythia oil showed 100% fumigant activity 24 h after treatment. Moreover, camphor, artemisia annua, eucalyptus, mugwort, and turpentine oils achieved 100% mortality 12 h after treatment at 5 mg/centrifuge tube to major workers. At a concentration of 5 mg/centrifuge tube, wintergreen, chrysanthemum, and forsythia oils showed 100% fumigant activity 24 h after treatment on major workers. Plant essential oils with >80% mortality at 5 mg/filter paper were observed at lower concentrations. Camphor, artemisia annua, and eucalyptus oils caused 100% mortality of minor workers at a concentration of 2 mg/centrifuge tube. In addition, wintergreen, mugwort, turpentine, and forsythia oils caused >80% mortality on minor workers at a concentration of 2 mg/centrifuge tube. For major workers, only camphor and eucalyptus oils caused >80% mortality at a concentration of 2 mg/centrifuge tube. After 24 h exposure to essential oils of camphor, artemisia annua, eucalyptus, and wintergreen at 1 mg/centrifuge tube, the insect mortalities were 100%, 100%, 80.00 ±6.67%, and 86.67 ±3.85% for minor workers and 60.00 ±6.67%, 60.00 ±6.67%, 53.33 ±6.67%, and 53.33

Table 2. Mortality of minor workers of red imported fire ants caused by essential oils at different concentrations (mg/centr. tube) in the fumigation bioassay for 12. h and 24 h.

essential oil	mg/centrifuge tube	Mortality (%,mean±SEM,N=3)* 12h	24h
camphor	5	100.00 a	100.00 a
	3	100.00 a	100.00 a
	2	100.00 a	100.00 a
	1	100.00 a	100.00 a
	0.5	51.11±5.88 efghi	86.67±7.70 bc
artemisia annua	5	100.00 a	100.00 a
	3	100.00 a	100.00 a
	2	100.00 a	100.00 a
	1	80.00±10.18 cd	100.00 a
	0.5	42.22±2.22 ghijkl	55.56±2.22 efgh
eucalyptus	5	100.00 a	100.00 a
	3	100.00 a	100.00 a
	2	100.00 a	100.00 a
	1	62.22±5.88 ef	80.00±6.67 cd
	0.5	35.56±2.22 ijklm	48.89±2.22 efghijk
wintergreen	5	66.67±7.70 de	100.00 a
	3	53.33±6.67 efghi	100.00 a
	2	33.33±3.85 jklm	100.00 a
	1	15.56±4.44 nop	86.67±3.85 c
	0.5	0.00 r	26.67±6.67 lmn
mugwort	5	100 a	100.00 a
	3	84.44±2.22 c	100.00 a
	2	66.67±3.85d e	86.67±10.18 bc
	1	31.11±2.22 klm	53.33±3.85 efghi
	0.5	13.33±3.85 nopq	24.44±2.22 mno
chrysanthemum	5	53.33±6.67 efghi	100.00 a
	3	33.33±6.67 klm	93.33±6.67 ab
	2	26.67±3.85 lmn	66.67±3.85 de
	1	6.67±3.85 q	26.67±7.70 lmn
	0.5	0.00 r	13.33±3.85 nopq
turpentine	5	100 a	100.00 a
	3	53.33±7.70 efghi	100.00 a
	2	40.00±3.85 hijklm	80.00±7.70 cd
	1	6.67±0.00 pq	40.00±3.85 hijklm
	0.5	0.00 r	6.67±3.85 q
forsythia	5	66.67±6.67 de	100.00 a
	3	46.67±7.70 fghijk	100.00 a
	2	33.33±3.85 jklm	86.67±3.85 c
	1	13.33±6.67 opq	60.00±7.70 efg
	0.5	0.00 r	0.00 r
ck	0	0.00 o	0.0 o

* Means sharing the same letters are not significantly different from each other (P>0.05, Duncan test).

±6.67% for major workers, respectively. However, these values decreased to 86.67 ±7.70%, 55.56 ±2.22%, 48.89 ±2.22%, and 26.67 ±6.67% for minor workers and 13.33 ±3.85%, 33.33 ±3.85%, 40.00 ±6.67%, and 20.00 ±6.67% for major workers, respectively, when the dosage decreased to 0.5 mg/centrifuge tube.

The toxicities of camphor, artemisia annua, eucalyptus, wintergreen, and mugwort oils after treatment on minor and major workers at a concentration of 2 mg/centrifuge tube were able to control the red imported fire ants (Figs. 1 and 2). Fig. 1 showed that camphor, artemisia annua, and eucalyptus oils completely killed the minor workers after 11 h, whereas wintergreen and mugwort oils showed 26.67 ± 3.85% and 46.67 ± 6.67% mortality, respectively. However, when the exposure time increased to 24 h, the wintergreen oil caused 100% mortality to the fire ants. Fig. 2 showed that the mortalities of major workers of red imported fire ant caused by camphor, eucalyptus, mugwort, wintergreen, and artemisia annua oils after 11 h were 73.33 ± 10.18%, 73.33 ± 6.67%, 40.00 ± 6.67%, 20 ± 3.85%, and 20 ± 6.67%, respectively, at a concentration of 2 mg/centrifuge tube. By contrast, at an exposure time of 24 h, the mortalities from the above five essential oils were 100%, 80 ± 7.70%, 46.67 ± 7.70%, 53.33 ± 6.67%, and 60 ± 6.67%, respectively.

Table 3. Analysis of variance (three-way ANOVA) for main effects (oi l type, dosage and exposure time) on mortality of major workers, and interactions.

	Main effects			Interactions			
	O	D	T	O×D	O×T	D×T	O×D×T
df	7	4	1	28	7	4	28
F	298.284	826.704	289.027	29.917	37.522	6.672	9.074
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

0: oil type, D: dosage, T: exposure time (P=0.05; df=239).

Discussion

This study showed that the mortalities of the workers of red imported fire ant after treatment varied according to the classification of workers, oil type, dosage, and exposure time. Minor workers had a significantly higher mortality than major workers. The essential oils of camphor, artemisia annua, eucalyptus, mugwort, and wintergreen had the strong toxicity against red imported fire ants at a low concentration ($P < 0.05$). In addition, camphor, artemisia annua, and eucalyptus oils had a higher activity than wintergreen and mugwort oils ($P < 0.05$). However, the active ingredient of the oils necessitates further studies.

Essential oils, widely used as fragrances and flavors in the perfume and food industries, have long been reputed to repel, contact, fumigate insects, and control some important plant pathogens (Koul et al., 2008). But the fumigant

Table 4. Mortality of major workers of red imported fire ants caused by essential oils at different concentrations (mg/centr. tube) in the fumigation bioassay for 12 h and 24 h.

essential oil	mg/centrifuge tube	Mortality (%,mean±SEM,N=3)*	
		12h	24h
camphor	5	100.00 a	100.00 a
	3	100.00 a	100.00 a
	2	73.33±7.70 cd	100.00 a
	1	53.33±7.70 efg	60.00±6.67 def
	0.5	6.67±3.85 n	13.33±3.85 lmn
artemisia annua	5	100.00 a	100.00 a
	3	93.33±7.70 b	100.00 a
	2	53.33±6.67 efg	73.33±6.67 cd
	1	40.00±3.85 ghi	60.00±6.67 def
	0.5	13.33±3.85 lmn	33.33±3.85 hijk
eucalyptus	5	100.00 a	100.00 a
	3	100.00 a	100.00 a
	2	73.33±6.67 cd	84.44±4.44 c
	1	40.00±6.67 ghi	53.33±6.67 efg
	0.5	13.33±3.85 lmn	40.00±6.67 ghi
wintergreen	5	26.67±7.70 ijkl	100.00 a
	3	20.00±3.85 jklm	100.00 a
	2	13.33±3.85 lmn	66.67±6.67 de
	1	6.67±3.85 n	53.33±6.67 efg
	0.5	0.00 o	20.00±6.67 jklm
mugwort	5	100.00 a	100.00 a
	3	66.67±10.18 de	100.00 a
	2	40.00±6.67 ghi	46.67±7.70 fgh
	1	26.67±7.70 ijkl	35.56±2.22 ghij
	0.5	6.67±3085 n	13.33±3.85 lmn
chrysanthemum	5	53.33±6.67efg	100.00 a
	3	33.33±3.85 hijk	40.00±6.67 ghi
	2	13.33±3.85 lmn	20.00±6.67 klm
	1	6.67±3.85 n	11.11±2.22 mn
	0.5	0.00 o	0.00 o
turpentine	5	100.00 a	100.00 a
	3	0.00 o	6.67±3.85 n
	2	0.00 o	0.00 o
	1	0.00 o	0.00 o
	0.5	0.00 o	0.00 o
forsythia	5	26.67±3.85 ijkl	100.00 a
	3	6.67±3.85 n	13.33±3.85 lmn
	2	0.00 o	0.00 o
	1	0.00 o	0.00 o
	0.5	0.00 o	0.00 o
ck	0	0.00 o	0.0 o

* Means sharing the same letters are not significantly different from each other ($P > 0.05$, Duncan test).

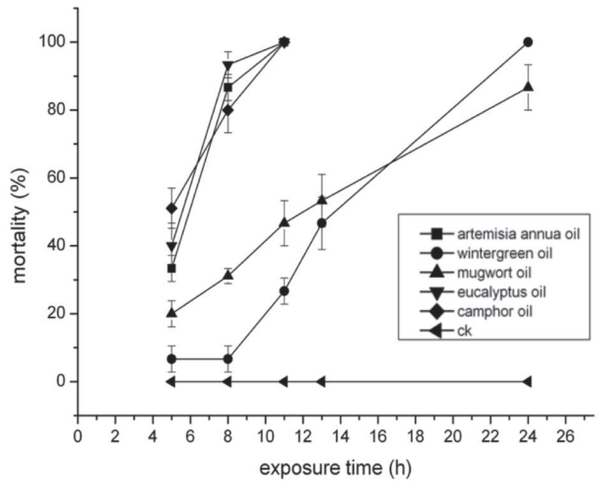

Figure 1. Mortality of minor workers of red imported fire ants treated with essential oils at 2 mg/centrifuge tube.

activities of essential oils against red imported fire ant were rarely reported. Zhou et al., (2011) tested the fumigant activity of methyl bromide and found that all red imported fire ant that were treated with methyl bromide above 22.52 g/m^2 for 8 h died. Plant essential oils from 29 plant species and components from garlic and glove bud oils, and plant essential oils from 30 plant species and components from ajowan, allspice, caraway, dill, geranium, and litsea oils were tested for their insecticidal activities against the Japanese termite, *R. speratus* Kolbe, by using a fumigation bioassay (Park & Shin 2005; Seo et al., 2009). Formic acid has been tested in the laboratory for contact and fumigation toxicity against workers, alates, and queens of red *S. invicta* (Chen, 2012).

The essential oils of camphor, artemisia annua, eucalyptus, mugwort, turpentine wintergreen, chrysanthemum, and forsythia showed effective toxicity against both minor and major workers of red imported fire ants. More specifically, camphor, artemisia annua, eucalyptus, mugwort, and wintergreen oils showed the strongest toxicity against red imported fire ants ($P < 0.05$).

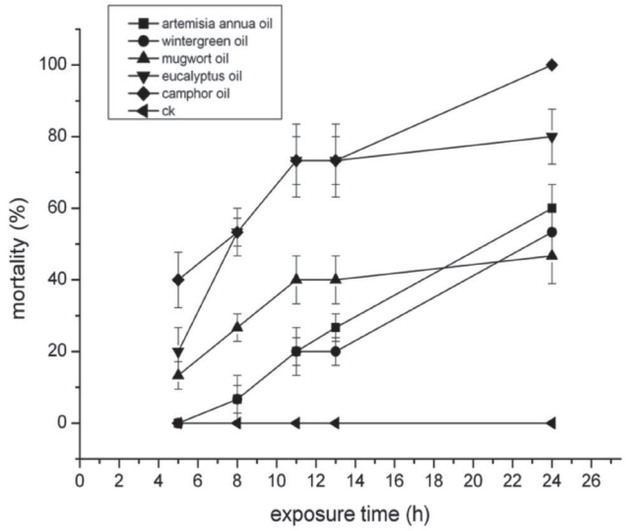

Figure 2. Mortality of major workers of red imported fire ants treated with essential oils at 2 mg/centrifuge tube .

As a conclusion, this study showed that camphor, artemisia annua, eucalyptus, mugwort, and wintergreen oils may have potential as natural fumigants, and can be used as substitutes for chemical insecticides. Moreover, the development of formulations with improved efficacy, stability and reduced costs necessitates further studies.

Acknowledgments

This study was supported by the Science and Technology Planning Project of Guangdong Province, China (No.2012B020309004).

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